

REPLACED BY  
ART 34 AMDT

Claims

1. A method of affinity separation wherein the affinity ligand is an immobilised proteinaceous ligand wherein one or more of its asparagine (Asn) residues has been modified.
2. A method of stabilising an affinity ligand by modifying one or more of its Asn residues.
3. A method of preparing a combinatorial library of protein molecules wherein the protein has been rendered less sensitive to alkaline pH by modification of one or more of its Asn residues before it is randomised.
4. A method of phage display wherein a protein expressed on the phage surface has had one or more of its Asn residues modified in a step separate to any modifications introduced in order to modify binding characteristics of the protein.
5. A method of making a stabilised combinatorial protein comprising the steps of:
  - a) modification of Asn residues within a protein molecule to increase stability of the protein in alkaline conditions; and
  - b) randomisation of the protein molecule to modify its binding characteristics.
6. A combinatorial protein wherein in a step separate from the randomisation step, the stability of the protein in alkaline conditions has been increased by modifying one or more of its Asn residues.
7. A fusion protein comprising a first part wherein one or more naturally occurring Asn residues have been modified and a second part being a randomised protein

molecule selected for its specific binding properties.

8. Use of a protein molecule stabilised by modification of one or more of its Asn residues in surface display or in affinity chromatography.

9. A method or a protein or a use as claimed in any one of claims 1 to 8, wherein one or more Asn residues in said ligand or said protein are replaced with a less alkaline-sensitive amino acid.

10. A method or a protein or a use as claimed in any one of claims 1 to 9, wherein two or more Asn residues are modified.

11. A method or a protein or a use as claimed in any one of claims 1 to 10, wherein all the Asn residues are modified.

12. A method or a protein or a use as claimed in any one of claims 1 to 11, wherein Asn residues on the surface of the three-dimensional structure of the ligand or protein are modified.

13. A method or a protein or a use as claimed in any one of claims 1 to 12, wherein said Asn residues are replaced with an amino acid selected from lysine, aspartic acid and leucine.

14. A method as claimed in any one of claims 1, 2 or 9 to 13, wherein said affinity ligand is a combinatorial protein.

15. A method as claimed in claim 14, wherein said affinity ligand is a randomised protein selected by expression in a surface display library.

16. A method or protein as claimed in any one of claims 6, 7, 14 or 15, wherein said combinatorial protein is derived from an immunoglobulin molecule or a fragment or derivative thereof, staphylococcal protein A (SPA) or a fragment, domain or derivative thereof, or a DNA binding protein, or fragment or domain thereof.

17. A method or protein as claimed in claim 16, wherein said combinatorial protein is domain Z, or a derivative thereof.

18. A method or protein or use as claimed in any one of claims 1 to 17, wherein said affinity ligand or protein is Albumin-Binding Protein (ABD) or a fragment or derivative thereof.

19. Albumin Binding Protein (ABD) or fragments or derivatives thereof wherein one or more native Asn residue have been replaced by a less alkaline sensitive amino acid.

20. A nucleic acid molecule encoding a protein as defined in any one of claims 6, 7, 9 to 13 or 16 to 19.

21. A host cell expressing a protein as defined in any one of claims 6, 7, 9 to 13 or 16 to 19.

22. A fusion protein as claimed in claim 7 wherein the first part is ABD and the second part is domain Z or a derivative thereof.